

Title: Development and Application of a Culture Method for Detection of *Helicobacter pylori* in Groundwater

Project I.D.: DNR Project #167

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Background/Need: *Helicobacter pylori* is recognized by the World Health Organization to be the primary cause of peptic ulcers, chronic gastritis and stomach cancer. About 50% of the U.S. population are thought to be symptomatic or asymptomatic carriers, even though the source of human infection is not well understood. One of the problems in understanding the source of human contamination is the difficulty in isolating the organism from the environment. Previously reported isolation techniques such as immuno-fluorescent microscopy or polymerase chain reaction (PCR) are problematic in that non-viable/non-infectious organisms will be detected, and the assays are costly. This project focused on the development of a culture media that selects viable *H. pylori* from samples containing mixed microbial populations, and thus enables routine screening of environmental samples for the presence of viable cells.

Objectives: The main objectives of this project were to create a culture method for selecting *Helicobacter pylori* from a heterogeneous microbial population in water, and then use this method to establish a data base for its occurrence in Wisconsin groundwaters.

Methods and Results:

- 1. Develop a collection of *H. pylori* isolates for use in experiments.**
Frozen stocks of *H. pylori* were stored in sufficient quantity to complete the entire study. The use of original frozen stock for each experiment eliminated the behavior variability sometimes seen in *H. pylori* strains that undergo multiple passes in laboratory conditions.
- 2. Screen and evaluate culture media.**
A selective media was developed which nurtured the growth of *Helicobacter* while inhibiting high populations of background contaminants. A color indicator was added to the media which enabled the technician to more easily differentiate *H. pylori* from non-target strains, and detect growth of the organism earlier.
- 3. Evaluate membrane filter concentration.**
Filter concentration of *H. pylori* was possible in volumes up to 1 liter but we did not pursue this method because of difficulty in detecting colonies grown on filters. Alternatively, a gravity (centrifuge) concentration of water samples was evaluated and implemented.
- 4. Evaluate enrichment and intra-cellular growth of *H. pylori* in ameba.**
The “Trojan horse” phenomenon of ameba engulfing and therefore protecting *H. pylori* from detrimental environmental conditions was investigated. However, the difficulty of culturing *H. pylori* in liquid media (also reported by Jiang & Doyle, 2000, J. Clin. Micro.; Kaspar, C.W., 2001, personal correspondence) made the coexistence of amoebas and bacteria impossible. Thus, validating the “Trojan horse” theory was not feasible.

5. Evaluate immuno-magnetic separation (IMS) techniques for isolating *H. pylori* from complex water matrix samples.

H. pylori could readily be recovered from water samples using immunomagnetic separation. However, enumeration of the target organism was not possible because the large beads allow attachment of multiple *H. pylori* cells per each bead and a bead-to-cell ratio could not be established. Thus, while the IMS method was effective in retrieving target cells from turbid, particle-laden samples, it could not be used for enumerating the captured organisms.

6. Screen Wisconsin well waters for the presence of *H. pylori*.

About 425 Wisconsin private wells, including a subset linked to people diagnosed with chronic gastritis, were tested for the presence of *H. pylori*. Wells were distributed across the entire state, and the quality of water ranged from clear and sterile to unsafe and turbid. Results from the plating method were confirmed by auxiliary use of immunomagnetic separation and fluorescent antibody staining. No *H. pylori* was found in any of the samples tested, and the authors conclude that Wisconsin well water collected in late winter/early spring is not a likely vector for the transmission of *H. pylori* to humans.

**Conclusions/
Implications:**

Prior to this study, there were no reliable methods for detecting viable *H. pylori* in environmental samples (water, manure, vegetables, etc.). Several research groups reported the presence of *Helicobacter* in the environment but the genomic and/or immunological methods used could not distinguish viable from nonviable cells. The efforts of this study resulted in the development of a high quality plating media for selecting viable *H. pylori* from mixed microbial populations. It also produced data that showed over 400 private wells, some related to infected residents, were *H. pylori*-absent. These results, as supported by (IMS) and/or fluorescent antibody (FA) assays, suggest that the route of *H. pylori* to humans in Wisconsin probably does not involve private well water.

Recommendations: This new plating media continues to draw requests for drinking water screening from Wisconsin residents suffering from chronic gastric distress. Also, the U.W. Gastroenterology Department has shown interest in using HP agar to isolate viable cells from biopsy specimens, and a Georgia Southern University researcher included this methodology in a grant proposal to the National Institute of Health. Other independent research groups reporting (non-published) the recovery of *H. pylori* from surface waters in high numbers have indicated interested in collaborating with the Wisconsin State Laboratory of Hygiene on further research. The authors look forward to continued *Helicobacter* research and defining its behavior and subsequent control in the environment.

**Related
Publications:**

none

Key Words:

Helicobacter pylori, bacterial culture methods, ulcers, drinking water

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Final Report:

A final report containing more detailed information on this project is available for loan at the Water Resources Institute Library, University of Wisconsin - Madison, 1975 Willow Drive, Madison, Wisconsin 53706 (608) 262-3069.